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(54) Title: DENDRIMER PRECURSOR DYES FOR IMAGING

(57) Abstract: The sensitivity and specificity of the optical modality can be enhanced by the use of highly absorbing dyes as contrast agents. Novel cyanine dyes that absorb and emit light in the near infrared region of electromagnetic spectrum are disclosed. These dyes are useful for imaging, diagnosis and therapy of various diseased states. Particularly, the molecules of the invention are useful for optical diagnostic imaging and therapy, in endoscopic applications for the detection of tumors and other abnormalities, for localized therapy, for photoacoustic tumor imaging, detection and therapy, and for sonofluorescence tumor imaging, detection and therapy.

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DENDRIMER PRECURSOR DYES FOR IMAGING

FIELD OF INVENTION

This invention relates generally to novel cyanine and indocyanine dyes for use
5 in imaging, diagnosis and therapy. Particularly, this invention relates to compositions
of cyanine and indocyanine dyes wherein novel carbocyclic and heterocyclic
moieties are incorporated into the polyene portion of the dye molecules.

BACKGROUND OF THE INVENTION

Several dyes that absorb and emit light in the visible and near-infrared region
10 of electromagnetic spectrum are currently being used for various biomedical
applications due to their biocompatibility, high molar absorptivity, or high
fluorescence quantum yields. The high sensitivity of the optical modality in
conjunction with dyes as contrast agents parallels that of nuclear medicine and
permits visualization of organs and tissues without the undesirable effect of ionizing
15 radiation. Cyanine dyes with intense absorption and emission in the near-infrared
(NIR) region are particularly useful because biological tissues are optically
transparent in this region (B. C. Wilson, Optical properties of tissues. *Encyclopedia
of Human Biology*, 1991, 5, 587-597). For example, indocyanine green, which
absorbs and emits in the NIR region has been used for monitoring cardiac output,
20 hepatic functions, and liver blood flow (Y-L. He, H. Tanigami, H. Ueyama, T.
Mashimo, and I. Yoshiya, Measurement of blood volume using indocyanine green
measured with pulse-spectrometry: Its reproducibility and reliability. *Critical Care
Medicine*, 1998, 26(8), 1446-1451; J. Caesar, S. Shaldon, L. Chiandussi, et al., The
use of Indocyanine green in the measurement of hepatic blood flow and as a test of
25 hepatic function. *Clin. Sci.* 1961, 21, 43-57) and its functionalized derivatives have

been used to conjugate biomolecules for diagnostic purposes (R. B. Mujumdar, L. A. Ernst, S. R. Mujumdar, et al., Cyanine dye labeling reagents: Sulfoindocyanine succinimidyl esters. *Bioconjugate Chemistry*, 1993, 4(2), 105-111; Linda G. Lee and Sam L. Woo. "N-Heteroaromatic ion and iminium ion substituted cyanine dyes for use as fluorescent labels", US 5,453,505; Eric Hohenschuh, et al. "Light imaging contrast agents", WO 98/48846; Jonathan Turner, et al. "Optical diagnostic agents for the diagnosis of neurodegenerative diseases by means of near infra-red radiation", WO 98/22146; Kai Licha, et al. "In-vivo diagnostic process by near infrared radiation", WO 96/17628; Robert A. Snow, et al., Compounds, WO 98/48838).

A major drawback in the use of cyanine dye derivatives is the potential for hepatobiliary toxicity resulting from the rapid clearance of these dyes by the liver (G. R. Cherrick, S. W. Stein, C. M. Leevy, et al., Indocyanine green: Observations on its physical properties, plasma decay, and hepatic extraction. *J. Clinical Investigation*, 1960, 39, 592-600). This is associated with the tendency of cyanine dyes to form aggregates in solution which could be taken up by Kupffer cells in the liver. Various attempts to obviate this problem have not been very successful. Typically, hydrophilic peptides, polyethyleneglycol or oligosaccharide conjugates have been used but these resulted in long-circulating products which are eventually cleared by the liver. Another major difficulty with current cyanine and indocyanine dye systems is that they offer a limited scope in the ability to induce large changes in the absorption and emission properties of these dyes. Attempts have been made to incorporate various heteroatoms and cyclic moieties into the polyene chain of these dyes (L. Strekowski, M. Lipowska, and G. Patonay, Substitution reactions of a nucleofugal group in hetamethine cyanine dyes. *J. Org. Chem.*, 1992, 57, 4578-

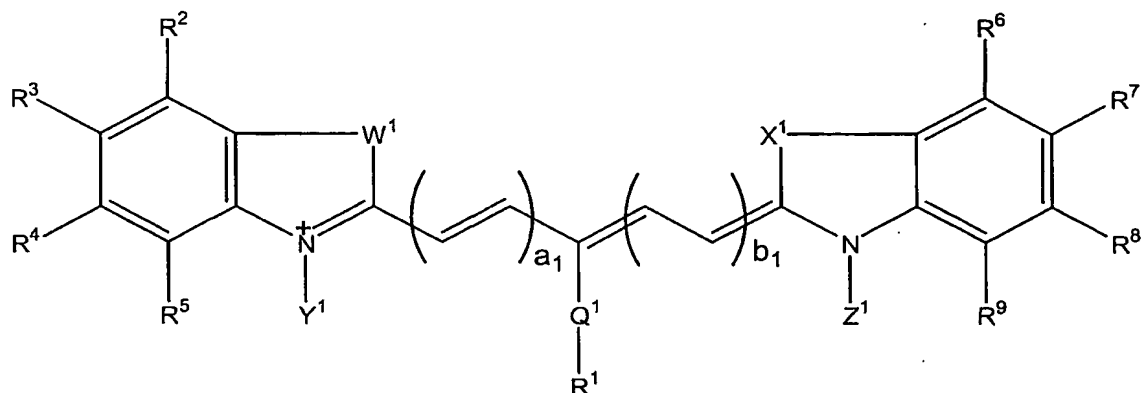
4580; N. Narayanan, and G. Patonay, A new method for the synthesis of heptamethine cyanine dyes: Synthesis of new near infrared fluorescent labels. *J. Org. Chem.*, 1995, 60, 2391-2395; E. Fung and R. Rajagopalan, Monocyclic functional dyes for contrast enhancement in optical imaging, US 5, 732,104; R. Rajagopalan and E. Fung, Delta^{1,6} bicyclo[4,4,0] functional dyes for contrast enhancement in optical imaging, US 5, 672, 333; R. Rajagopalan and E. Fung, Tricyclic functional dyes for contrast enhancement in optical imaging, US 5, 709, 845) but the resulting dye systems do not show large differences in absorption and emission maxima, especially beyond 830 nm where photacoustic diagnostic applications are very sensitive. They also possess prominent hydrophobic core which enhances liver uptake. Further, most cyanine dyes do not have the capacity to form dendrimers which are useful in biomedical applications.

Therefore, there is a need to design novel dyes that could prevent dye aggregation in solution, predisposed to form dendrimers , capable of absorbing or emitting beyond 800 nm, possess desirable photophysical properties, and endowed with tissue-specific targeting capability.

The publications and other materials used herein to support the background of the invention or provide additional details respecting the practice, are incorporated by reference.

SUMMARY OF THE INVENTION

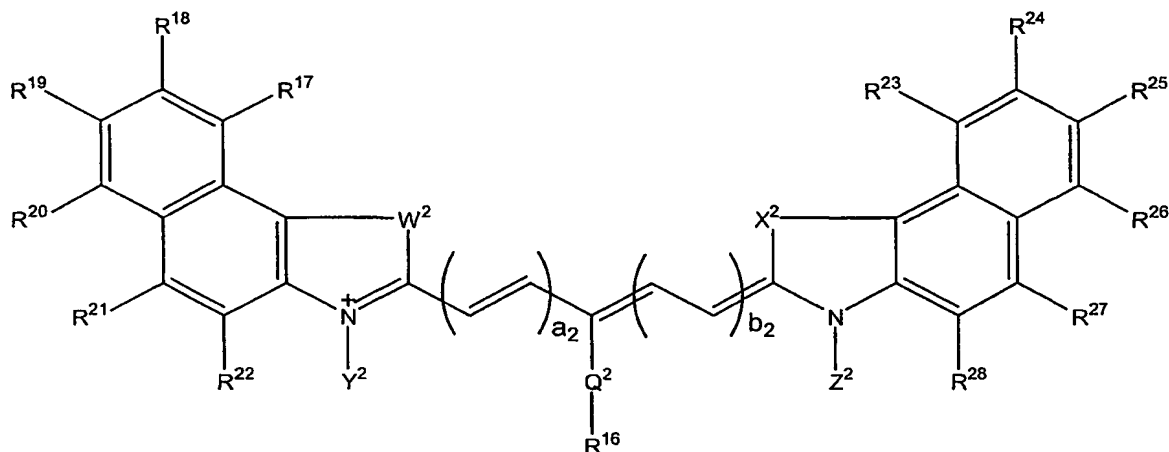
The present invention relates particularly to the novel composition comprising cyanine dyes of general formula 1



Formula 1

- 5 wherein a_1 and b_1 vary from 0 to 5; W^1 and X^1 may be the same or different and are selected from the group consisting of $-CR^{10}R^{11}$, $-O-$, $-NR^{12}$, $-S-$, and $-Se$; Q^1 is a single bond or is selected from the group consisting of $-O-$, $-S-$, $-Se-$, and $-NR^{13}$; Y^1 and Z^1 may be the same or different and are selected from the group consisting of $-(CH_2)_c-CO_2H$, $-CH_2-(CH_2-O-CH_2)_d-CH_2-CO_2H$, $-(CH_2)_e-NH_2$, $-CH_2-(CH_2-O-CH_2)_f-CH_2-$
- 10 NH_2 , $-(CH_2)_g-N(R^{14})-(CH_2)_h-CO_2H$, and $-(CH_2)_i-N(R^{15})-CH_2-(CH_2-O-CH_2)_j-CH_2-CO_2H$; R^1 and R^{10} to R^{15} may be same or different and are selected from the group consisting of $-hydrogen$, $C1-C10$ alkyl, $C1-C10$ aryl, $C1-C10$ alkoxy, $C1-C10$ polyalkoxyalkyl, $-CH_2(CH_2-O-CH_2)_c-CH_2-OH$, $C1-C20$ polyhydroxyalkyl, $C1-C10$ polyhydroxyaryl, $-(CH_2)_d-CO_2H$, $-CH_2-(CH_2-O-CH_2)_e-CH_2-CO_2H$, $-(CH_2)_fNH_2$, and $-$
- 15 $CH_2-(CH_2-O-CH_2)_g-CH_2-NH_2$; c , e , g , h , and i vary from 1 to 10; d , f and j vary from 1 to 100; and R^2 to R^9 may be the same or different and are selected from the group consisting of $hydrogen$, $C1-C10$ alkyl, $C1-C10$ aryl, $hydroxyl$, $C1-C10$ polyhydroxyalkyl, $C1-C10$ alkoxy, $amino$, $C1-C10$ aminoalkyl, $cyano$, $nitro$ and $halogen$.

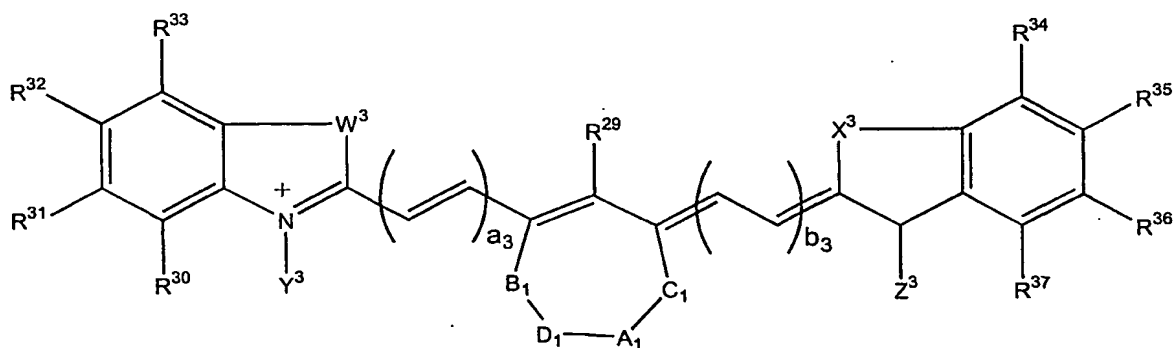
The present invention also relates to the novel composition comprising
indocyanine dyes of general formula 2



Formula 2

5 wherein a_2 and b_2 are defined in the same manner as a_1 and b_1 ; W^2 and X^2 are defined in the same manner W^1 and X^1 ; Q^2 is defined in the same manner as Q^1 ; R^{16} and R^{10} to R^{15} are defined in the same manner as R^1 and R^{10} to R^{15} ; Y^2 is defined in the same manner as Y^1 ; Z^2 is defined in the same manner as Z^1 ; and R^{17} to R^{28} are defined in the same manner as R^2 to R^9 .

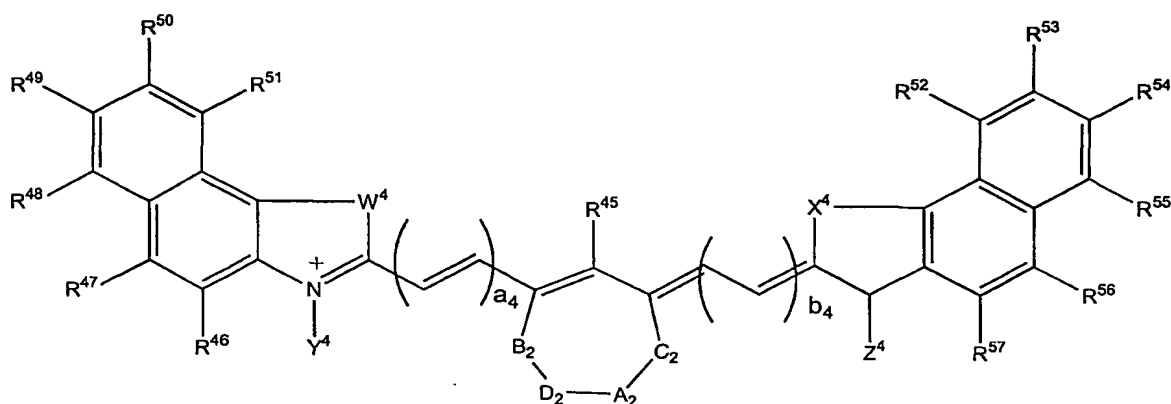
10 The present invention also relates to the novel composition comprising
cyanine dyes of general formula 3



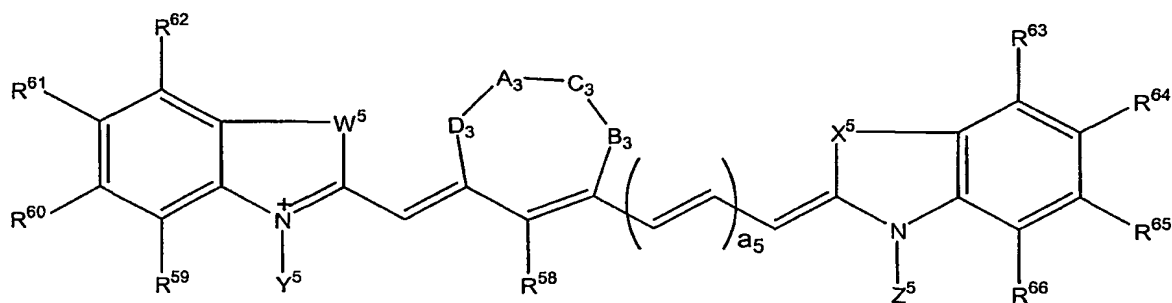
Formula 3

wherein a_3 and b_3 are defined in the same manner as a_1 and b_1 ; W^3 and X^3 are defined in the same manner W^1 and X^1 ; Y^3 is defined in the same manner as Y^1 ; Z^3 is defined in the same manner as Z^1 ; A_1 is a single or a double bond; if A_1 is a single bond, then B_1 and C_1 may be the same or different and are selected from the group consisting of -O-, -S-, -Se-, -P-, and -NR³⁸ and D_1 is selected from the group consisting of -CR³⁹R⁴⁰, and -C=O; if A_1 is a double bond, then B_1 is selected from the group consisting of -O-, -S-, -Se-, -P- and -NR³⁸, C_1 is nitrogen or -CR⁴¹, and D_1 is -CR⁴²; R^{29} to R^{37} are selected from the group consisting of hydrogen, C1-C10 alkyl, C1-C10 aryl, hydroxyl, hydrophilic peptide, C1-C10 polyhydroxyalkyl, C1-C10 alkoxy, cyano, nitro, halogen and -NR⁴³R⁴⁴; R^{38} to R^{42} may be same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_c-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; c, e, g, h, and i vary from 1 to 10; d, f and j vary from 1 to 100; R^{43} and R^{44} may be the same or different and are selected from the group consisting of hydrogen, C1-C10 alkyl, C1-C10 aryl, or may together form a 5, 6, or 7 membered carbocyclic ring or a 5, 6, or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or a sulfur atom.

The present invention also relates to the novel composition comprising indocyanine dyes of general formula 4 wherein a_4 and b_4 are defined in the same manner as a_1 and b_1 ; W^4 and X^4 are defined in the same manner as W^1 and X^1 ; Y^4 is defined in the same manner as Y^1 ; Z^4 is defined in the same manner as Z^1 ; A_2 is defined in the same manner as A_1 ; B_2 , C_2 , and D_2 are defined in the same manner as B_1 , C_1 , and D_1 ; and R^{45} to R^{57} are defined in the same manner as R^{29} to R^{37} .

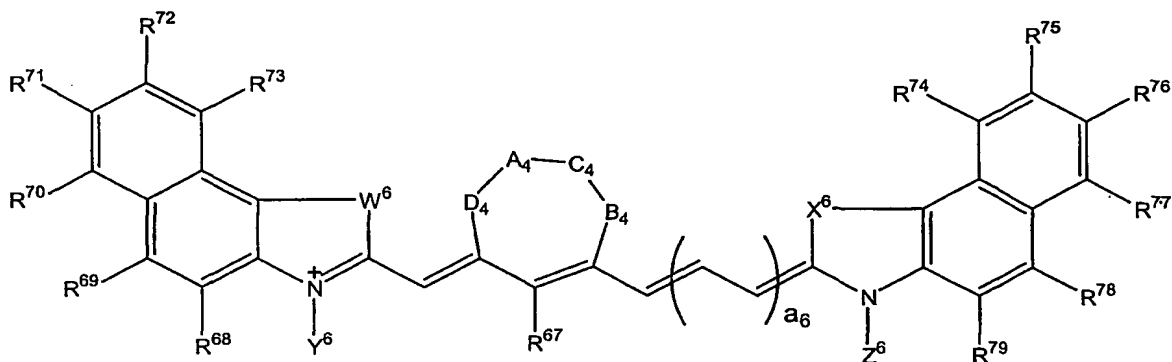
**Formula 4**

The present invention also relates to the novel composition comprising cyanine dyes of general formula 5

**Formula 5**

wherein a_5 is defined in the same manner as a_1 ; W^5 and X^5 are defined in the same manner W^1 and X^1 ; Y^5 is defined in the same manner as Y^1 ; Z^5 is defined in the same manner as Z^1 ; A_3 is defined in the same manner as A_1 ; B_3 , C_3 , and D_3 are defined in the same manner as B_1 , C_1 , and D_1 ; and R^{58} to R^{66} are defined in the same manner as R^{29} to R^{37} .

The present invention also relates to the novel composition comprising cyanine dyes of general formula 6



Formula 6

5 wherein a_6 is defined in the same manner as a_1 ; W^6 and X^6 are defined in the same manner as W^1 and X^1 ; Y^6 is defined in the same manner as Y^1 ; Z^6 is defined in the same manner as Z^1 ; A_4 is defined in the same manner as A_1 ; B_4 , C_4 , and D_4 are defined in the same manner as B_1 , C_1 , and D_1 ; and R^{67} to R^{79} are defined in the same manner as R^{29} to R^{37} .

10 This invention is also related to the method of conjugating the dyes of this invention to peptides or biomolecules by solid phase synthesis.

This invention is also related to the method of preventing fluorescence quenching. It is known that cyanine dyes generally form aggregates in aqueous media leading to fluorescence quenching. We observed that in instances where the presence of hydrophobic core in the dyes lead fluorescence quenching, the addition of a biocompatible organic solvent such as 1-50% dimethylsulfoxide (DMSO) restored the fluorescence by preventing aggregation and allowed in vivo organ
15 visualization.

20 DETAILED DESCRIPTION OF THE INVENTION

The novel compositions of the present invention comprising dyes of formulas 1 to 6 offer significant advantages over those currently described in the art. As

illustrated in Schemes 1-6, these dyes are designed to prevent aggregation in solution by preventing intramolecular and intermolecular ordered hydrophobic interactions. They also have multiple attachment sites proximal to the dye chromophore for ease of forming dendrimers. The presence of rigid and extended chromophore backbone enhances fluorescence quantum yield and extends the maximum absorption beyond 800 nm. Conjugation of biomolecules to these dyes are readily achievable. They are useful in various biomedical applications including, but not limited to, tomographic imaging of organs; monitoring of organ functions; coronary angiography; fluorescence endoscopy; detection, imaging, and therapy of tumors; laser guided surgery, photoacoustic and sonofluorescence methods; and the like. Specific embodiments to accomplish some of the aforementioned biomedical applications are given below.

In one embodiment of the invention, the dyes of the invention are useful for optical tomographic, endoscopic, photoacoustic and sonofluorescence applications for the detection and treatment of tumors and other abnormalities.

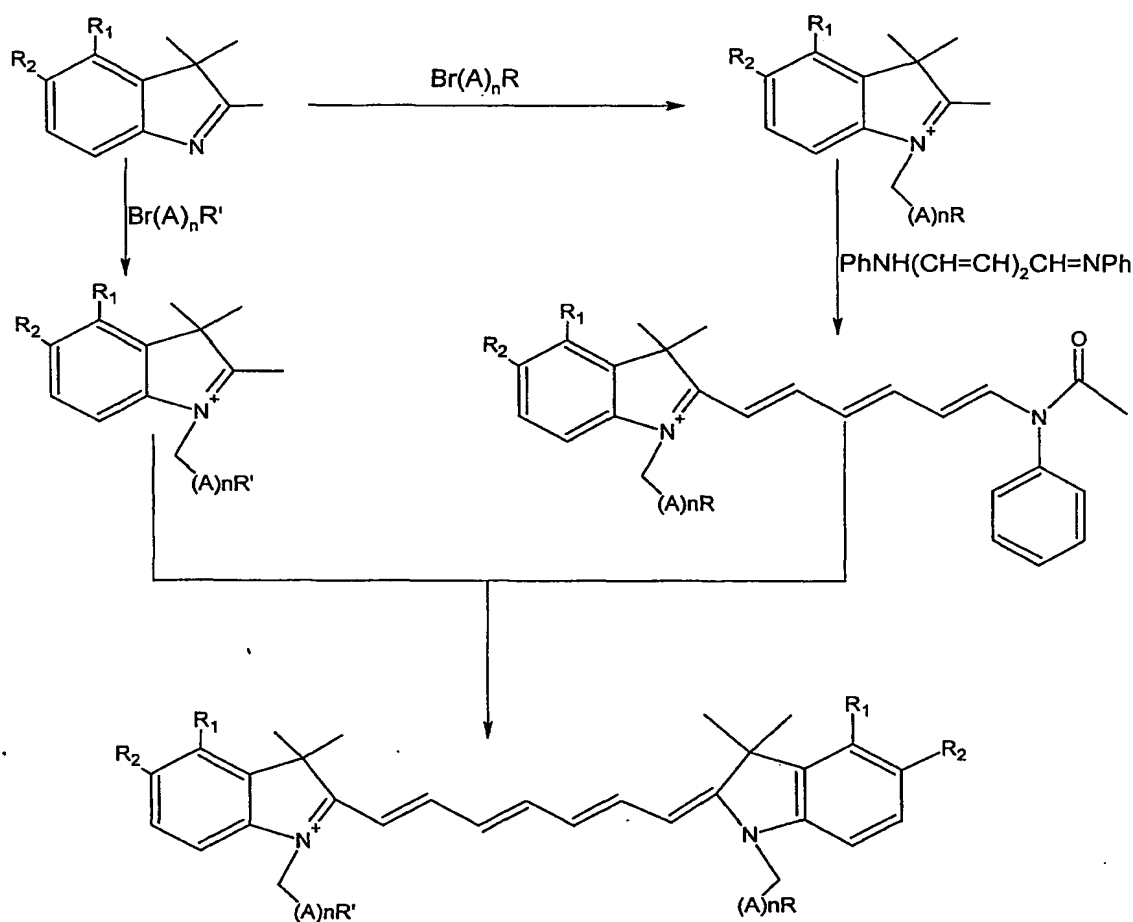
In another aspect of the invention, the dyes of the invention are useful for localized therapy.

In yet another aspect of the invention, the dyes of the invention are useful for the detection of the presence of tumors and other abnormalities by monitoring the blood clearance profile of the dyes.

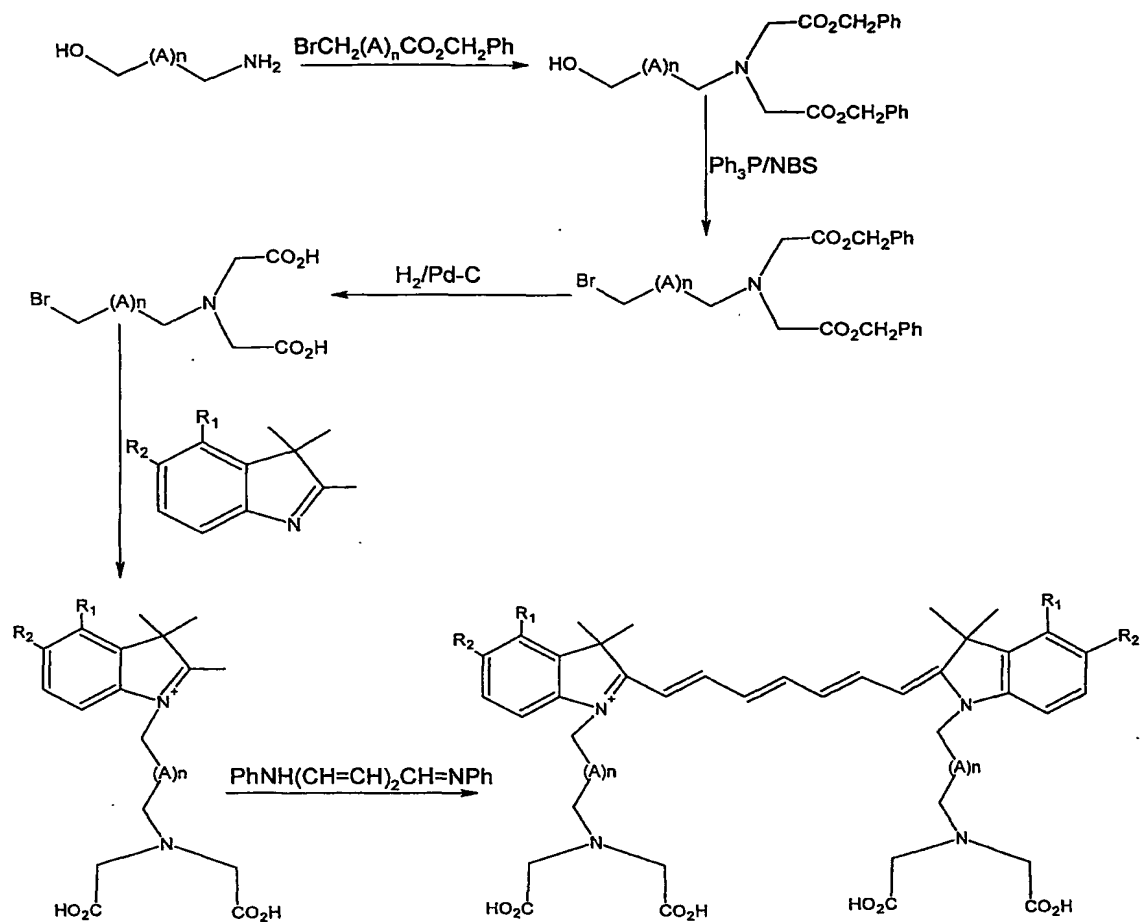
In a further embodiment of the invention, the dyes are useful for laser assisted guided surgery for the detection of micrometastases of tumors upon laparoscopy.

In yet another aspect of the invention, the dye bioconjugates of the dyes of this invention are useful diagnosis of atherosclerotic plaques and blood clots.

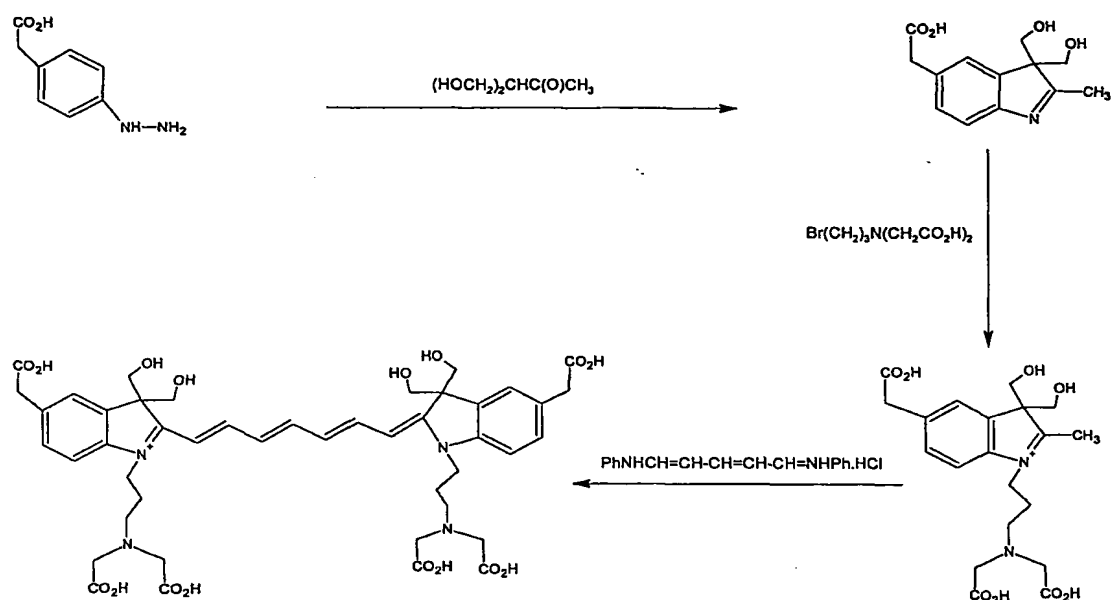
The novel dyes of the present invention are prepared according the methods well known in the art and are shown in Schemes 1-5 and their use in the synthesis of bioconjugates is shown in Scheme 6.



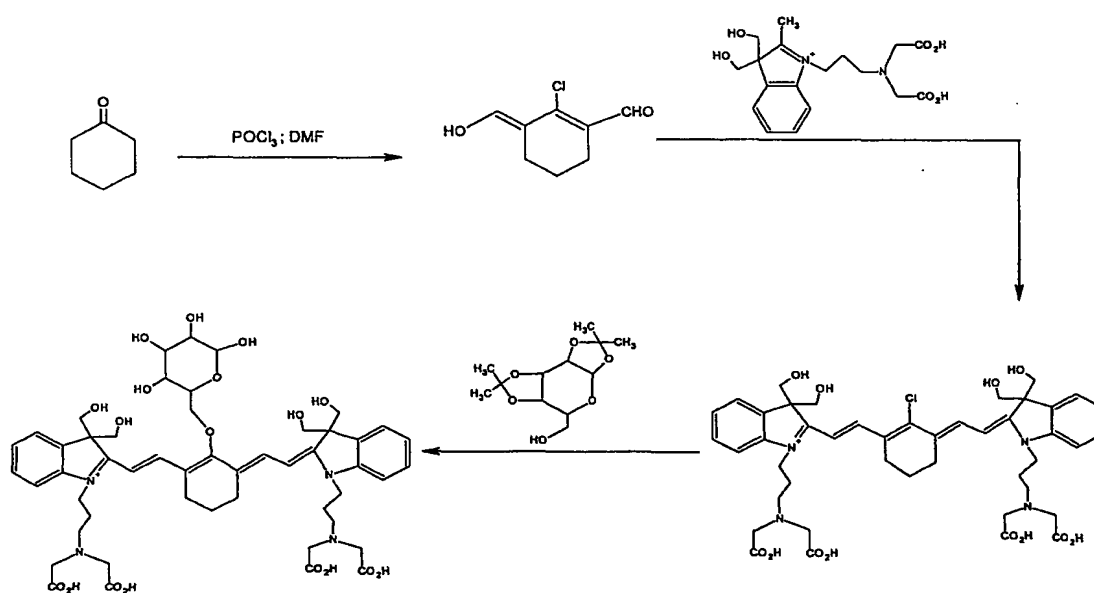
Scheme 1: Preparation of bis-carboxylates ; $A = CH_2$ or CH_2OCH_2 ;
 $R = COOH$; $R' = COOH, NHFmoc, CO_2t-Bu, SO_3^-$
 $R_1 = R_2 = H$ (Formula 1) or $R_1, R_2 =$ fused phenyl (Formula 2)



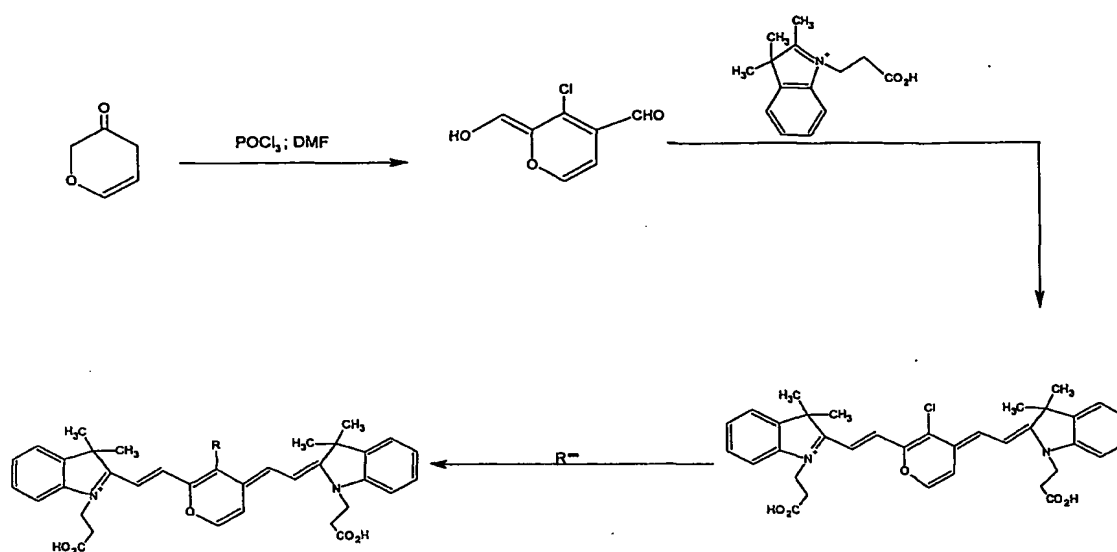
Scheme 2: Preparation of Tetra-carboxylates ; A = CH₂ or CH₂OCH₂;
 R₁ = R₂ = H (Formula 1) or R₁, R₂ = fused phenyl (Formula 2)



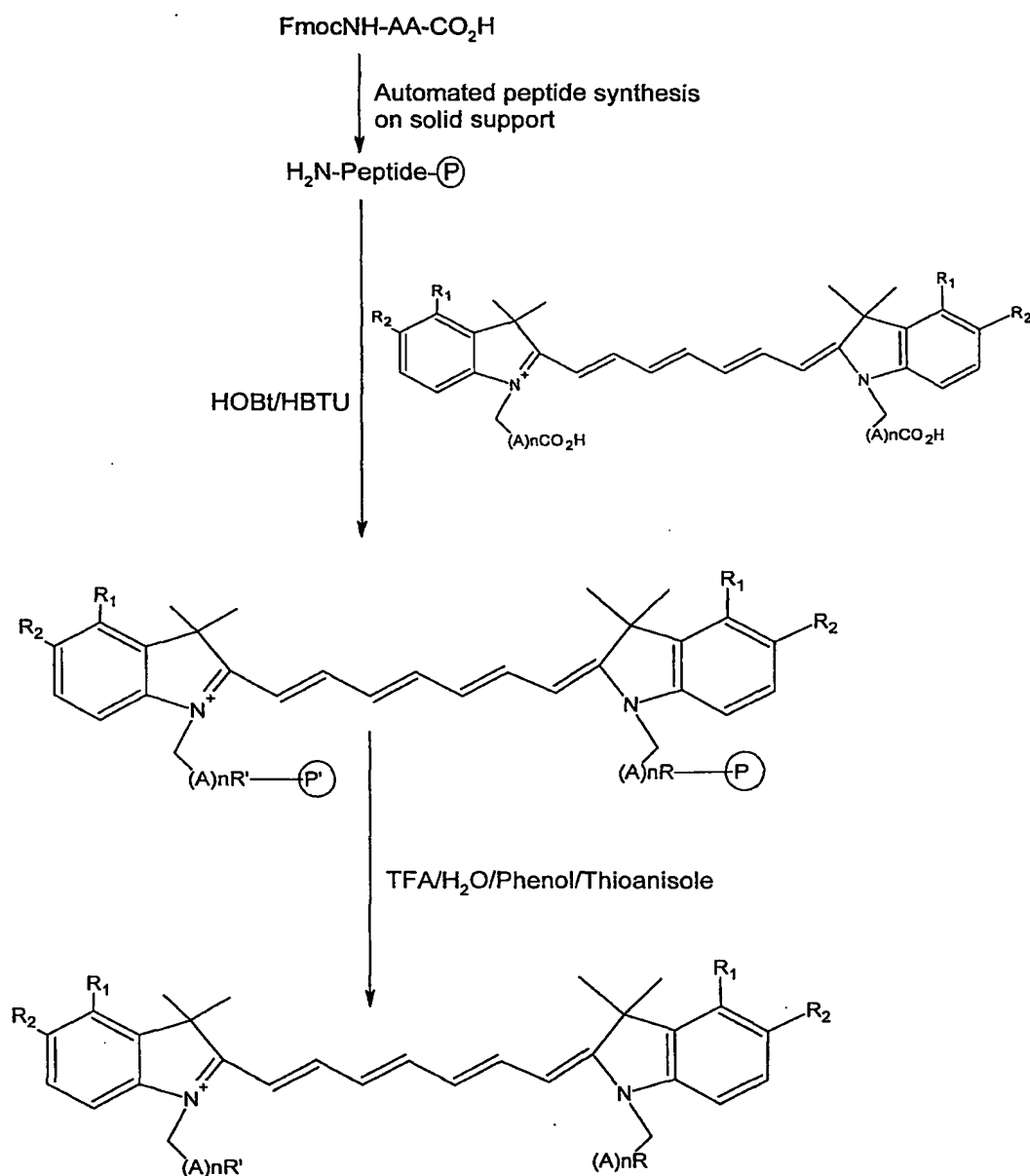
Scheme 3: Preparation of polyhydroxy-carboxylic acid cyanine dyes



Scheme 4: Synthesis of non-aggregating cyanine dyes



Scheme 5: Synthesis of "tunable" cyanine dyes



Scheme 6: Representative scheme for the preparation of bioconjugates ;A = CH₂ or CH₂OCH₂; R₁ = R₂ = H (Formula 1) or R₁, R₂ = fused phenyl (Formula 2); AA = Amino Acids; R = CONH peptide; R' = R (Bis conjugate) or COOH (Mono conjugate)

(P) = Solid support; (P') = Presence or absence depends on R' definition;

In a preferred embodiment, the dyes according to the present invention have the general Formula 1 wherein a₁ and b₁ vary from 0 to 3; Q¹ is a single bond; R¹ to R⁹ are hydrogens; W¹ and X¹ may be the same or different and are selected from the

group consisting of $-\text{C}(\text{CH}_3)_2$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{OH})\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{OH})_2$,

$\text{C}((\text{CH}_2)_{\text{zz}}\text{CO}_2\text{H})\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{CO}_2\text{H})_2$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{NH}_2)\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{NH}_2)_2$,

$\text{C}((\text{CH}_2)_{\text{zz}}\text{NR}_{\text{pp}}\text{R}_{\text{pz}})\text{CH}_3$ and $\text{C}((\text{CH}_2)_{\text{zz}}\text{NR}_{\text{pp}}\text{R}_{\text{pz}})_2$; Y^1 and Z^1 may be the same or

different and are selected from the group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-}$

$\text{CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-}$

$\text{CH}_2\text{-CO}_2\text{H}$; R_{pp} and R_{pz} may be the same or different and are selected from the group

consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(CH}_2)_h\text{-}$

CO_2H , and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R^{14} and R^{15} may be same or

different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-

C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, $-\text{CH}_2\text{(CH}_2\text{-O-CH}_2)_c\text{-CH}_2\text{-OH}$, C1-

C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(\text{CH}_2)_d\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_e\text{-}$

$\text{CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, and $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_g\text{-CH}_2\text{-NH}_2$; c, e, g, h, i, and zz vary

from 1 to 5; and d, f and j vary from 1 to 100.

In another preferred embodiment, the dyes according to the present invention

have the general Formula 2 wherein a_2 and b_2 vary from 0 to 3; Q^2 is a single bond;

R^{16} to R^{28} are hydrogens; W^2 and X^2 may be the same or different and are selected

from the group consisting of $-\text{C}(\text{CH}_3)_2$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{OH})\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{OH})_2$,

$\text{C}((\text{CH}_2)_{\text{zz}}\text{CO}_2\text{H})\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{CO}_2\text{H})_2$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{NH}_2)\text{CH}_3$, $\text{C}((\text{CH}_2)_{\text{zz}}\text{NH}_2)_2$,

$\text{C}((\text{CH}_2)_{\text{zz}}\text{NR}_{\text{pp}}\text{R}_{\text{pz}})\text{CH}_3$ and $\text{C}((\text{CH}_2)_{\text{zz}}\text{NR}_{\text{pp}}\text{R}_{\text{pz}})_2$; Y^2 and Z^2 may be the same or

different and are selected from the group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-}$

$\text{CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-}$

$\text{CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R_{pp} and R_{pz} may be the same or different and are selected from the

group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(}$

$\text{CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R^{14} and R^{15} may be

same or different and are selected from the group consisting of -hydrogen, C1-C10

alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, $-\text{CH}_2(\text{CH}_2\text{-O-CH}_2)_c\text{-CH}_2\text{-OH}$, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(\text{CH}_2)_d\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_e\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, and $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_g\text{-CH}_2\text{-NH}_2$; c, e, g, h, i, and zz vary from 1 to 5; and d, f and j vary from 1 to 100.

- 5 In another preferred embodiment, the dyes according to the present invention have the general Formula 3 wherein a_3 and b_3 vary from 0 to 3; A_1 is a single bond; B_1 is selected from the group consisting of -O-, -S-, and $-\text{NR}^{38}$; C_1 is $-\text{CH}_2$ or $-\text{C=O}$; D_1 is selected from the group consisting of -O-, -S-, and $-\text{NR}^{38}$; R^{29} is a hydrogen, a halogen atom, a saccharide or a hydrophilic peptide; R^{30} to R^{37} are hydrogens; R^{38}
- 10 is selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, $-\text{CH}_2(\text{CH}_2\text{-O-CH}_2)_c\text{-CH}_2\text{-OH}$, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(\text{CH}_2)_d\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_e\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, and $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_g\text{-CH}_2\text{-NH}_2$; W^3 and X^3 may be the same or different and are selected from the group consisting of $-\text{C}(\text{CH}_3)_2$,
- 15 $\text{C}((\text{CH}_2)_{zz}\text{OH})\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{OH})_2$, $\text{C}((\text{CH}_2)_{zz}\text{CO}_2\text{H})\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{CO}_2\text{H})_2$, $\text{C}((\text{CH}_2)_{zz}\text{NH}_2)\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{NH}_2)_2$, $\text{C}((\text{CH}_2)_{zz}\text{NR}_{pp}\text{R}_{pz})\text{CH}_3$ and $\text{C}((\text{CH}_2)_{zz}\text{NR}_{pp}\text{R}_{pz})_2$; Y^3 and Z^3 may be the same or different and are selected from the group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R_{pp} and R_{pz} may be the same or different
- 20 and are selected from the group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N(R}^{14})\text{-(CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R^{14} and R^{15} may be same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, $-\text{CH}_2(\text{CH}_2\text{-O-CH}_2)_c\text{-CH}_2\text{-OH}$, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(\text{CH}_2)_d\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_e\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, and -
- 25

$\text{CH}_2-(\text{CH}_2-\text{O}-\text{CH}_2)_g-\text{CH}_2-\text{NH}_2$; c, e, g, h, i, and zz vary from 1 to 5; and d, f and j vary from 1 to 100.

In another preferred embodiment, the dyes according to the present invention have the general Formula 4 wherein a_4 and b_4 vary from 0 to 3; A_2 is a single or
 5 double bond; B_2 is selected from the group consisting of -O-, -S-, or -NR³⁸; C_2 is -CH₂ or -C=O; D_2 is selected from the group consisting of -O-, -S- and -NR³⁸; R³⁸ is selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_c-CH₂-OH, C1-C20
 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-
 10 CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; R⁴⁵ is a hydrogen, a halogen atom, a saccharide or a hydrophilic peptide; R⁴⁶ to R⁵⁷ are hydrogens; W⁴ and X⁴ may be the same or different and are selected from the group consisting of -C(CH₃)₂,
 , C((CH₂)_{zz}OH)CH₃, C((CH₂)_{zz}OH)₂, ((CH₂)_{zz}CO₂H)CH₃, C((CH₂)_{zz}CO₂H)₂,
 C((CH₂)_{zz}NH₂)CH₃, C((CH₂)_{zz}NH₂)₂, C((CH₂)_{zz}NR_{pp}R_{pz})CH₃ and C((CH₂)_{zz}NR_{pp}R_{pz})₂;
 15 Y⁴ and Z⁴ may be the same or different and are selected from the group consisting of
 -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, and -
 (CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_f-CH₂-CO₂H; R_{pp} and R_{pz} are the same or different and
 are selected from -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-
 (CH₂)_h-CO₂H, and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_f-CH₂-CO₂H; R¹⁴ and R¹⁵ may be
 20 the same or different and are selected from the group consisting of -hydrogen, C1-
 C10 alkyl, C1-C10 aryl, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_c-
 CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-
 (CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; c, e, g, h, i,
 and zz vary from 1 to 5; and d, f and j vary from 1 to 100.

In another preferred embodiment, the dyes according to the present invention have the general Formula 5 wherein a_5 varies from 0 to 3; A_3 is a single or double bond; B_3 is selected from the group consisting of -O-, -S- and -NR³⁸; C_3 is -CH₂ or -C=O; D_3 is selected from the group consisting of -O-, -S- and -NR³⁸; R³⁸ is selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_e-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; R⁵⁸ is a hydrogen, a halogen atom, a saccharide or a hydrophilic peptide; R⁵⁹ to R⁶⁶ are hydrogens; W⁵ and X⁵ may be the same or different and are selected from the group consisting of -C(CH₃)₂, C((CH₂)_{zz}OH)CH₃, C((CH₂)_{zz}OH)₂, C((CH₂)_{zz}CO₂H)CH₃, C((CH₂)_{zz}CO₂H)₂, C((CH₂)_{zz}NH₂)CH₃, C((CH₂)_{zz}NH₂)₂, C((CH₂)_{zz}NR_{pp}R_{pz})CH₃ and C((CH₂)_{zz}NR_{pp}R_{pz})₂; Y⁵ and Z⁵ may be the same or different and are selected from the group consisting of -(CH₂)_e-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; R_{pp} and R_{pz} are the same or different and are selected from -(CH₂)_e-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; R¹⁴ and R¹⁵ may be the same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_e-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; c, e, g, h, i, and zz vary from 1 to 5; and d, f and j vary from 1 to 100.

In another preferred embodiment, the dyes according to the present invention have the general Formula 6 wherein a_6 varies from 0 to 3; A_4 is a single or double bond; B_4 is selected from the group consisting of -O-, -S- and -NR³⁸; C_4 is -CH₂ or

-C=O; D_4 is selected from the group consisting of -O-, -S- and -NR³⁸; R³⁸ is selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_c-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂,
 5 and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; R⁶⁷ is a hydrogen, a halogen atom, a saccharide or a hydrophilic peptide; R⁶⁸ to R⁷⁹ are hydrogens; W⁶ and X⁶ may be the same or different and are selected from the group consisting of -C(CH₃)₂, C((CH₂)_{zz}OH)CH₃, C((CH₂)_{zz}OH)₂, C((CH₂)_{zz}CO₂H)CH₃, C((CH₂)_{zz}CO₂H)₂, C((CH₂)_{zz}NH₂)CH₃, C((CH₂)_{zz}NH₂)₂, C((CH₂)_{zz}NR_{pp}R_{pz})CH₃ and C((CH₂)_{zz}NR_{pp}R_{pz})₂; Y⁶ and Z⁶ may be
 10 the same or different and are selected from the group consisting of -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; R_{pp} and R_{pz} are the same or different and are selected from the group consisting of -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; R¹⁴ and R¹⁵ may be
 15 the same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_c-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂; c, e, g, h, i, and zz vary from 1 to 5; and d, f and j vary from 1 to 100.

20 The compositions of the invention can be formulated into diagnostic compositions for enteral or parenteral administration. These compositions contain an effective amount of the dye along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of dye
 25 according to this invention. Parenteral compositions may be injected directly or

mixed with a large volume parenteral composition for systemic administration. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride.

Formulations for enteral administration may vary widely, as is well known in the art. In general, such formulations are liquids which include an effective amount of the dye in aqueous solution or suspension. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement. Such doses may vary widely; depending upon the particular dye employed, the organs or tissues which are the subject of the imaging procedure, the imaging equipment being used, and the like.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blooded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure.

A combination of the above represents an important approach to the synthesis and use of novel cyanine and indocyanine dyes with a variety of photophysical and chemical properties. The dyes of this invention are new and they are useful for biomedical applications. The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the scope of the invention in any manner. Reliable computational methods for the prediction of the absorption maxima of some of the dyes were also established.

Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1

5 Synthesis of Bis(ethylcarboxymethyl)indocyanine Dye

(Scheme 1, R₁, R₂ = fused phenyl; A = CH₂, n = 1 and R = R' = CO₂H)

A mixture of 1,1,2-trimethyl-[1H]-benz[e]indole (9.1 g, 43.58 mmoles) and 3-bromopropanoic acid (10.0 g, 65.37 mmoles) in 1,2-dichlorobenzene (40 mL) was heated at 110 °C for 12 hours. The solution was cooled to room temperature and the
10 red residue obtained was filtered and washed with acetonitrile:diethyl ether (1:1) mixture. The solid obtained was dried under vacuum to give 10 g (64%) of light brown powder. A portion of this solid (6.0 g; 16.56 mmoles), glutaconaldehyde dianil monohydrochloride (2.36 g, 8.28 mmoles) and sodium acetate trihydrate (2.93 g, 21.53 mmoles) in ethanol (150 mL) were refluxed for 90 minutes. After evaporating
15 the solvent, 40 mL of a 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the supernatant became nearly colorless. About 5 mL of water:acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain 2 g of dark green flakes. The purity of the compound was established with ¹H-NMR and LC-Mass
20 spectrometry.

EXAMPLE 2

Synthesis of Bis(pentylcarboxymethyl)indocyanine Dye

(Scheme 1, R₁, R₂ = fused phenyl; A = CH₂, n = 4 and R = R' = CO₂H)

A mixture of 1,1,2-trimethyl-[1H]-benz[e]indole (20 g, 95.6 mmol) and 6-bromohexanoic acid (28.1 g, 144.1 mmol) in 1,2-dichlorobenzene (250 mL) was heated at 110 °C for 12 hours. The green solution was cooled to room temperature and the brown solid precipitate formed was collected by filtration. After washing the solid with 1,2-dichlorobenzene and diethyl ether, the brown powder obtained (24 g, 64%) was dried under vacuum at room temperature. A portion of this solid (4.0 g; 9.8 mmol), glutaraldehyde dianil monohydrochloride (1.4 g, 5 mmol) and sodium acetate trihydrate (1.8 g, 12.9 mmol) in ethanol (80 mL) were refluxed for 1 hour. After evaporating the solvent, 20 mL of a 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the supernatant became nearly colorless. About 5 mL of water:acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain about 2 g of dark green flakes. The purity of the compound was established with ¹H-NMR and LC-Mass spectrometry.

EXAMPLE 3

Synthesis of Bisethylcarboxymethylindocyanine Dye

(Scheme 1, R₁ = R₂ = H; A = CH₂, n = 1 and R = R' = CO₂H)

This compound was prepared as described in Example 1 except that 1,1,2-trimethylindole was used as the starting material.

EXAMPLE 4

Synthesis of Bis(hexaethyleneglycolcarboxymethyl)indocyanine Dye

(Scheme 1, R₁ = R₂ = fused phenyl; A = CH₂OCH₂, n = 6 and R = R' = CO₂H)

This compound was prepared as described in Example 1 except that ω -bromohexaoxyethyleneglycolpropionic acid was used in place of bromopropanoic acid and the reaction was carried out in 1,2-dimethoxypropane.

5 EXAMPLE 5

Synthesis of Bisethylcarboxymethylindocyanine Dye

(Scheme 2. $R_1 = R_2 =$ fused phenyl; $A = CH_2$, and $n = 0$)

A solution of 50 ml of dimethylformamide and benzyl bromoacetate (16.0 g, 70 mmol) was stirred in a 100 ml three-neck flask. Solid potassium bicarbonate (7.8 g, 78 mmol) was added. The flask was purged with argon and cooled to 0°C with an ice bath. To the stirring mixture was added dropwise a solution of ethanolamine (1.9 g, 31 mmol) and 4 ml of dimethylformamide over 5 minutes. After the addition was complete the mixture was stirred for 1 hour at 0°C. The ice bath was removed and the mixture stirred at room temperature over night. The reaction mixture was partitioned between 100 ml of methylene chloride and 100 ml of saturated sodium bicarbonate solution. The layers were separated and the methylene chloride layer was again washed with 100 ml of saturated sodium bicarbonate solution. The combined aqueous layers were extracted twice with 25 ml of methylene chloride. The combined methylene chloride layers were washed with 100 ml of brine, and dried over magnesium sulfate. The methylene chloride was removed with aspirator vacuum at ca. 35°C, and the remaining dimethylformamide was removed with vacuum at about 45°C. The crude material was left on a vacuum line over night at room temperature.

The crude material from above was dissolved in 100 ml of methylene chloride at room temperature. Triphenylphosphine (8.91 g, 34 mmol) was added and dissolved with stirring. An argon purge was started and the mixture cooled to 0°C with an ice bath. The N-bromosuccinimide (6.05 g, 34 mmol) was added portionwise over 2 minutes. The mixture was stirred for 1.5 hours at 0°C. The methylene chloride was removed with vacuum and gave a purple oil. This oil was triturated with 200 ml of ether with constant manual stirring. During this time the oil became very thick. The ether solution was decanted and the oil was triturated with 100 ml of

ether. The ether solution was decanted and the oil was again triturated with a 100 ml portion of ether. The ether was decanted and the combined ether solutions allowed to stand for about 2 hours to allow the triphenylphosphine oxide to crystallize. The ether solution was decanted from the crystals and the solid washed with 100 ml of ether. The volume of the combined ether abstracts was reduced with vacuum until a volume of about 25 ml was obtained. This was allowed to stand over night at 0°C. Ether (10 ml) was added to the cold mixture which was mixed to suspend the solid. The mixture was percolated through a column of 45 g of silica gel and eluted with ether, 75 ml fractions were collected. The fractions that contained product by TLC were pooled and the ether removed with vacuum. This gave 10.1 g of crude product. The material was flash chromatographed on silica gel with hexane, changing to 9:1 hexane:ether. The product-containing fractions were pooled and the solvents removed with vacuum. This gave 7.4 g (57% yield) of pure product.

A mixture of 10% palladium on carbon (1 g) and a solution of the benzyl ester (10 g) in 150 ml of methanol was hydrogenolyzed at 25 psi for 2 hours. The mixture was filtered over celite and the residue was washed with methanol. The solvent was evaporated to give a viscous oil in quantitative yield.

Reaction of the bromide with 1,1,2-trimethyl-[1H]-benz[e]indole was carried out as described in Example 1.

EXAMPLE 6

Bis(ethylcarboxymethyldihydroxyl)indocyanine Dye (Scheme 3)

The hydroxy-indole compound is readily prepared by literature method (P. L. Southwick, J. G. Cairns, L. A. Ernst, and A. S. Waggoner, One pot Fischer synthesis of (2,3,3-trimethyl-3-H-indol-5-yl)-acetic acid derivatives as intermediates for fluorescent biolabels. *Org. Prep. Proced. Int. Briefs*, 1988, 20(3), 279-284). Reaction of p-carboxymethylphenylhydrazine hydrochloride (30 mmol, 1 equiv.) and 1,1-bis(hydroxymethyl)propanone (45 mmole, 1.5 equiv.) in acetic acid (50 mL) at room temperature for 30 minutes and at reflux for 1 gives (3,3-dihydroxymethyl-2-methyl-3-

H-indol-5-yl)-acetic acid as a solid residue. The reaction of 3-bromopropyl-N,N-bis(carboxymethyl)amine, which was prepared as described in Example 5, with the intermediate indole and subsequent reaction of the indole intermediate with glutacanaldehyde dianil monohydrochloride (see Example 1.) gives the desired product.

EXAMPLE 7

Synthesis of Bis(propylcarboxymethyl)indocyanine Dye (Scheme 4)

The intermediate 2-chloro-1-formyl-3-hydroxymethylenecyclohexane was prepared as described in the literature (G. A. Reynolds and K. H. Drexhage, Stable heptamethine pyrylium dyes that absorb in the infrared. *J. Org. Chem.*, 1977, 42(5), 885-888). Equal volumes (40 mL each) of dimethylformamide (DMF) and dichloromethane were mixed and the solution was cooled to -10 °C in acetone-dry ice bath. Under argon atmosphere, phosphorus oxychloride (40 mL) in dichloromethane was added dropwise to the cool DMF solution. The resulting solution was allowed to warm up to room temperature and refluxed for 6 hours. After cooling to room temperature, the mixture was poured into ice-cold water and stored at 4 °C for 12 hours. About 8g of yellow powder was obtained after filtration. Condensation of the cyclic dialdehyde with the indole intermediate is carried out as described in Example 1. Further the functionalization of the dye with bis (isopropylidene acetal protected monosaccharide by the method described in the literature (J. H. Flanagan, C. V. Owens, S. E. Romero, et al., Near infrared heavy-atom-modified fluorescent dyes for base-calling in DNA-sequencing application using temporal discrimination. *Anal. Chem.*, 1998, 70(13), 2676-2684).

EXAMPLE 8

Synthesis of Bis(ethylcarboxymethyl)indocyanine Dye (Scheme 5)

These dyes are prepared as described in Experiment 7. These dyes absorb in
5 the infrared region. The typical example shown in Scheme 5 have estimated
absorption maximum at 1036 nm.

EXAMPLE 9

Synthesis of Peptides

10 The procedure described below is for the synthesis of Octreotate. Other
peptides were prepared by a similar procedure with minor modifications in some
cases. These peptides were used to illustrate the ease of using dyes of this invention
to prepare bioconjugates.

15 The octapeptide was prepared by an automated fluorenylmethoxycarbonyl
(Fmoc) solid phase peptide synthesis using a commercial peptide synthesizer from
Applied Biosystems (Model 432A SYNERGY Peptide Synthesizer). The first peptide
cartridge contained Wang resin pre-loaded with Fmoc-Thr on a 25 μ mole scale.
Subsequent cartridges contained Fmoc-protected amino acids with side chain
20 protecting groups for the following amino acids: Cys(Acm), Thr(t-Bu), Lys(Boc),
Trp(Boc) and Tyr(t-Bu). The amino acid cartridges were placed on the peptide
synthesizer and the product was synthesized from the C- to the N-terminal position.
The coupling reaction was carried out with 75 μ moles of the protected amino acids in
the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium
25 hexafluorophosphate (HBTU)/N-hydroxybenzotriazole (HOBt). The Fmoc protecting

group was removed with 20% piperidine in dimethylformamide. After the synthesis was complete, the thiol group was cyclized with thallium trifluoroacetate and the product was cleaved from the solid support with a cleavage mixture containing trifluoroacetic acid (85%):water (5%):phenol (5%):thioanisole (5%) for 6 hours. The peptide was precipitated with t-butyl methyl ether and lyophilized with water:acetonitrile (2:3) mixture. The peptide was purified by HPLC and analyzed with LC/MS. The amino acid sequence of Octreotate is: D-Phe-Cys'-Tyr-D-Trp-Lys-Thr-Cys'-Thr, wherein Cys' indicates the presence of an intramolecular disulfide bond between two cysteine amino acids.

Octreotide was prepared by the same procedure : D-Phe-Cys'-Tyr-D-Trp-Lys-Thr-Cys'-Thr-OH, wherein Cys' indicates the presence of an intramolecular disulfide bond between two cysteine amino acids.

Bombesin analogs were prepared by the same procedure except that cyclization with thallium trifluoroacetate was not needed. Side-chain deprotection and cleavage from the resin was carried out with 50 μ L each of ethanedithiol, thioanisole and water, and 850 μ L of trifluoroacetic acid . Two analogues were prepared: Gly-Ser-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH₂ and Gly-Asp-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH₂.

Cholecystokinin octapeptide analogs were prepared as described for Octreotate without the cyclization step. Three analogs were prepared: Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂; Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH₂; D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH₂;

Neurotensin analog was prepared as described for Octreotate without the cyclization step: D-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu.

5 EXAMPLE 10

Synthesis of Peptide-Dye Conjugates

The method described below is for the synthesis of Octreotate conjugates but a similar procedure is used for the synthesis of other peptide-dye conjugates.

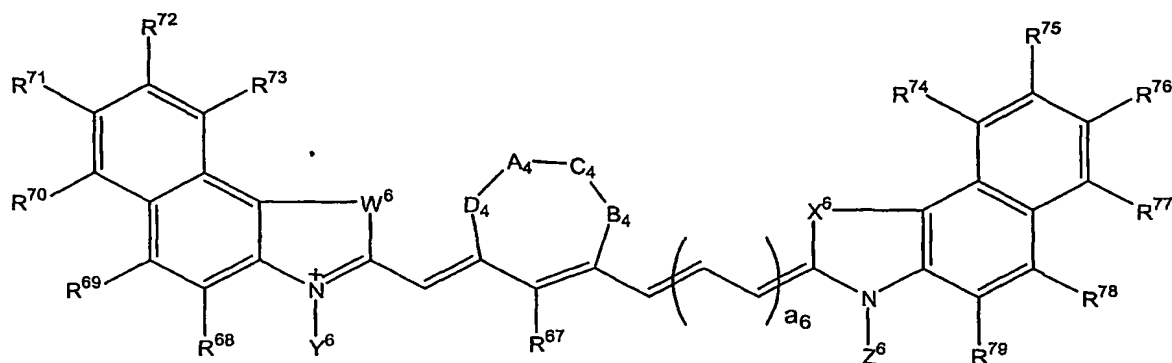
10 The Octreotate was prepared as described in Example 6 but the peptide was not cleaved from the solid support and the N-terminal Fmoc group of Phe was retained. The thiol group was cyclized with thallium trifluoroacetate and the Phe was deprotected to liberate the free amine. Bisethylcarboxymethylindocyanine dye (53 mg, 75 μ moles) was added to an activation reagent consisting of a 0.2 M solution of
15 HBTU/HOBt in DMSO (375 μ L), and 0.2 M solution of diisopropylethylamine in DMSO (375 μ L). The activation was complete in about 30 minutes and the resin-bound peptide (25 μ moles) was added to the dye. The coupling reaction was carried out at room temperature for 3 hours. The mixture was filtered and the solid residue was washed with DMF, acetonitrile and THF. After drying the green residue, the
20 peptide was cleaved from the resin and the side chain protecting groups were removed with a mixture of 85% trifluoroacetic acid, 2.5% water, 2.5% thioanisole and 2.5% phenol. The resin was filtered and cold t-butyl methyl ether (MTBE) was used to precipitate the dye-peptide conjugate which was dissolved in acetonitrile:water (2:3) mixture and lyophilized. The product was purified by HPLC to give the
25 monoOctreotate- Bisethylcarboxymethylindocyanine dye (**Cytate 1**, 80%) and the

bisOctreotate- Bisethylcarboxymethylindocyanine dye (**Cytate 2**, 20%). The monoOctreotate conjugate can be obtained almost exclusively (>95%) over the bis conjugate by reducing the reaction time to 2 hours. However, this also leads to incomplete reaction and the free Octreotate must be carefully separated from the dye conjugate in order to avoid saturation of the receptors by the non-dye conjugated peptide.

Octreotate-bispenylcarboxymethylindocyanine dye was prepared as described above with some modifications. Bispenylcarboxymethylindocyanine dye (60 mg, 75 μ moles) was added to an activation reagent consisting of a 0.2 M solution of HBTU/HOBt in DMSO (400 μ L), and 0.2 M solution of diisopropylethylamine in DMSO (400 μ L). The activation was complete in about 30 minutes and the resin-bound peptide (25 μ moles) was added to the dye. The reaction was carried out at room temperature for 3 hours. The mixture was filtered and the solid residue was washed with DMF, acetonitrile and THF. After drying the green residue, the peptide was cleaved from the resin and the side chain protecting groups were removed with a mixture of 85% trifluoroacetic acid, 2.5% water, 2.5% thioanisole and 2.5% phenol. The resin was filtered and cold t-butyl methyl ether (MTBE) was used to precipitate the dye-peptide conjugate which was dissolved in acetonitrile:water (2:3) mixture and lyophilized. The product was purified by HPLC to give Octreotate-1,1,2-trimethyl-[1H]-benz[e]indole propanoic acid conjugate (10%), monoOctreotate-bispenylcarboxymethylindocyanine dye (**Cytate 3**, 60%) and bisOctreotate-bispenylcarboxymethylindocyanine dye (**Cytate 4**, 30%).

While the invention has been disclosed by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the
5 scope of the appended claims.

1. A composition comprising indocyanine dyes of formula



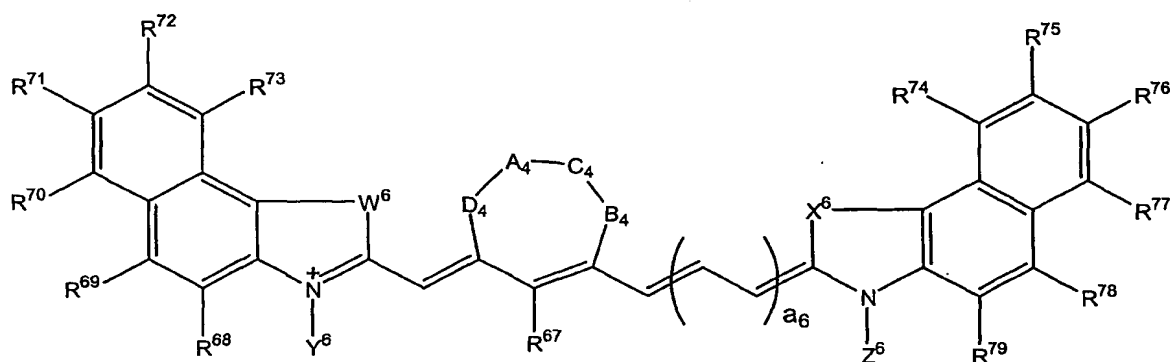
wherein a_6 varies from 0 to 5; W^6 and X^6 may be the same or different and are selected from the group consisting of $-CR^{10}R^{11}$, $-O-$, $-NR^{12}$, $-S-$, and $-Se$; Y^6 and Z^6 may be the same or different and are selected from the group consisting of $-(CH_2)_c-CO_2H$, $-CH_2-(CH_2-O-CH_2)_d-CH_2-CO_2H$, $-(CH_2)_e-NH_2$, $-CH_2-(CH_2-O-CH_2)_f-CH_2-NH_2$, $-(CH_2)_g-N(R^{14})-(CH_2)_h-CO_2H$, and $-(CH_2)_i-N(R^{15})-CH_2-(CH_2-O-CH_2)_j-CH_2-CO_2H$; A_4 is a single or a double bond; if A_4 is a single bond, then B_4 and C_4 may be the same or different and are selected from the group consisting of $-O-$, $-S-$, $-Se-$, $-P-$ and $-NR^{38}$ and D_4 is selected from $-CR^{39}R^{40}$ and $-C=O$; if A_4 is a double bond, then B_4 is selected from the group consisting of $-O-$, $-S-$, $-Se-$, $-P-$ and $-NR^{38}$, C_4 is selected from nitrogen and $-CR^{41}$ and D_4 is $-CR^{42}$; R^{67} to R^{79} may be the same or different and are selected from the group consisting of hydrogen, C1-C10 alkyl, hydrophilic peptide, C1-C10 aryl, hydroxyl, C1-C10 polyhydroxyalkyl, C1-C10 alkoxyl, cyano, nitro, halogen, and $-NR^{43}R^{44}$; R^{38} to R^{42} may be same or different and are selected from the group consisting of $-hydrogen$, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxyl, C1-C10 polyalkoxyalkyl, $-CH_2(CH_2-O-CH_2)_c-CH_2-OH$, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(CH_2)_d-CO_2H$, $-CH_2-(CH_2-O-CH_2)_e-CH_2-CO_2H$, $-(CH_2)_f-NH_2$, and $-CH_2-(CH_2-O-CH_2)_g-CH_2-NH_2$; c , e , g , h , and i vary from 1 to 10; d , f and j vary from 1 to 100; and R^{43} and R^{44} may be

the same or different and are selected from the group consisting of hydrogen, C1-C10 alkyl, and C1-C10 aryl, or may together form a 5, 6, or 7 membered carbocyclic ring or a 5,6, or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or sulfur atoms

- 5 2. The composition of claim 1 wherein a_6 varies from 0 to 3; A_4 is a single bond; B_4 is selected from the group consisting of -O-, -S-, and -NR³⁸; C_4 is selected from -CH₂ and -C=O; D_4 is selected from the group consisting of -O-, -S-, and -NR³⁸; R^{67} is selected from the group consisting of a hydrogen, a halogen atom, a saccharide and a hydrophilic peptide; R^{68} to R^{79} are hydrogens; W^6 and X^6 may
- 10 be the same or different and are selected from the group consisting of -C(CH₃)₂, C((CH₂)_{zz}OH)CH₃, C((CH₂)_{zz}OH)₂, C((CH₂)_{zz}CO₂H)CH₃, C((CH₂)_{zz}CO₂H)₂, C((CH₂)_{zz}NH₂)CH₃, C((CH₂)_{zz}NH₂)₂, C((CH₂)_{zz}NR_{pp}R_{pz})CH₃ and C((CH₂)_{zz}NR_{pp}R_{pz})₂; Y^6 and Z^6 may be the same or different and are selected from the group consisting of -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -
- 15 (CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; R_{pp} and R_{pz} may be the same or different and are selected from -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, and -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H; c, e, g, h, i, and zz vary from 1 to 5; d, f and j vary from 1 to 100; and R^{14} and R^{15} may be same or different and are selected from the
- 20 group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂-(CH₂-O-CH₂)_e-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂.
- 25 3. The composition of claim 1 wherein a_6 is 1; A_4 is a double bond; B_4 is -O-; C_4 and D_4 are CH; R^{67} is selected from the group consisting of hydrogen, saccharide and

hydrophilic peptide; R^{68} to R^{79} are hydrogens; W^6 and X^6 may be the same or different and selected from the group consisting of $-C((CH_2)_zOH)_2$, $C((CH_2)_zCO_2H)_2$ and $C((CH_2)_zNR_{pp}R_{pz})_2$; Y^6 and Z^6 are the same and are selected from the group consisting of $-(CH_2)_c-CO_2H$, $-CH_2-(CH_2-O-CH_2)_d-CH_2-CO_2H$ and $-(CH_2)_g-N(R^{14})-(CH_2)_h-CO_2H$.

4. A method for performing a diagnostic or therapeutic procedure which comprises administering to an individual an effective amount of the composition of indocyanine dyes of the formula



wherein a_6 varies from 0 to 5; W^6 and X^6 may be the same or different and are selected from the group consisting of $-CR^{10}R^{11}$, $-O-$, $-NR^{12}$, $-S-$, and $-Se$; Y^6 and Z^6 may be the same or different and are selected from the group consisting of $-(CH_2)_c-CO_2H$, $-CH_2-(CH_2-O-CH_2)_d-CH_2-CO_2H$, $-(CH_2)_e-NH_2$, $-CH_2-(CH_2-O-CH_2)_f-CH_2-NH_2$, $-(CH_2)_g-N(R^{14})-(CH_2)_h-CO_2H$, and $-(CH_2)_i-N(R^{15})-CH_2-(CH_2-O-CH_2)_j-CH_2-CO_2H$; A_4 is a single or a double bond; if A_4 is a single bond, then B_4 and C_4 may be the same or different and are selected from the group consisting of $-O-$, $-S-$, $-Se-$, $-P-$ and $-NR^{38}$ and D_4 is selected from $-CR^{39}R^{40}$ and $-C=O$; if A_4 is a double bond, then B_4 is selected from the group consisting of $-O-$, $-S-$, $-Se-$, $-P-$ and $-NR^{38}$, C_4 is selected from nitrogen and $-CR^{41}$ and D_4 is $-CR^{42}$; R^{67} to R^{79} may be the same or different and are selected from the group consisting of hydrogen, C1-

C10 alkyl, hydrophilic peptide, C1-C10 aryl, hydroxyl, C1-C10 polyhydroxyalkyl, C1-C10 alkoxy, cyano, nitro, halogen, and $-NR^{43}R^{44}$; R^{38} to R^{42} may be same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, $-\text{CH}_2(\text{CH}_2\text{-O-CH}_2)_c\text{-CH}_2\text{-}$
 5 OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, $-(\text{CH}_2)_d\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_e\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, and $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_g\text{-CH}_2\text{-NH}_2$; c, e, g, h, and i vary from 1 to 10; d, f and j vary from 1 to 100; and R^{43} and R^{44} may be the same or different and are selected from the group consisting of hydrogen, C1-C10 alkyl, and C1-C10 aryl, or may together form a 5, 6, or 7 membered
 10 carbocyclic ring or a 5,6, or 7 membered heterocyclic ring optionally containing one or more oxygen, nitrogen, or sulfur atoms.

5. The method of performing the diagnostic or therapeutic procedure of claim 4 which comprises administering to an individual an effective amount of the composition of indocyanine dyes wherein a_6 varies from 0 to 3; A_4 is a single
 15 bond; B_4 is selected from the group consisting of -O-, -S-, and $-NR^{38}$; C_4 is selected from $-\text{CH}_2$ and $-\text{C=O}$; D_4 is selected from the group consisting of -O-, -S-, and $-NR^{38}$; R^{67} is selected from the group consisting of hydrogen, a halogen atom, a saccharide and a hydrophilic peptide; R^{68} to R^{79} are hydrogens; W^6 and X^6 may be the same or different and are selected from the group consisting of -
 20 $\text{C}(\text{CH}_3)_2$, $\text{C}((\text{CH}_2)_{zz}\text{OH})\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{OH})_2$, $\text{C}((\text{CH}_2)_{zz}\text{CO}_2\text{H})\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{CO}_2\text{H})_2$, $\text{C}((\text{CH}_2)_{zz}\text{NH}_2)\text{CH}_3$, $\text{C}((\text{CH}_2)_{zz}\text{NH}_2)_2$, $\text{C}((\text{CH}_2)_{zz}\text{NR}_{pp}\text{R}_{pz})\text{CH}_3$ and $\text{C}((\text{CH}_2)_{zz}\text{NR}_{pp}\text{R}_{pz})_2$; Y^6 and Z^6 may be the same or different and are selected from the group consisting of $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-CH}_2)_d\text{-CH}_2\text{-CO}_2\text{H}$, $-(\text{CH}_2)_g\text{-N}(\text{R}^{14})\text{-(CH}_2)_h\text{-CO}_2\text{H}$, and $-(\text{CH}_2)_i\text{-N}(\text{R}^{15})\text{-CH}_2\text{-(CH}_2\text{-O-CH}_2)_j\text{-CH}_2\text{-CO}_2\text{H}$; R_{pp} and R_{pz}
 25 are the same or different and are selected from $-(\text{CH}_2)_c\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{-O-}$

CH₂)_d-CH₂-CO₂H, -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H, or -(CH₂)_i-N(R¹⁵)-CH₂-(CH₂-O-CH₂)_j-CH₂-CO₂H ; c, e, g, h, i, and zz vary from 1 to 5; d, f and j vary from 1 to 100; and R¹⁴ and R¹⁵ may be same or different and are selected from the group consisting of -hydrogen, C1-C10 alkyl, C1-C10 aryl, C1-C10 alkoxy, C1-C10 polyalkoxyalkyl, -CH₂(CH₂-O-CH₂)_e-CH₂-OH, C1-C20 polyhydroxyalkyl, C1-C10 polyhydroxyaryl, -(CH₂)_d-CO₂H, -CH₂-(CH₂-O-CH₂)_e-CH₂-CO₂H, -(CH₂)_f-NH₂, and -CH₂-(CH₂-O-CH₂)_g-CH₂-NH₂.

6. The method of performing the diagnostic or therapeutic procedure of claim 4 which comprises administering to an individual an effective amount of the composition of indocyanine dyes wherein a₆ is 1; A₄ is a double bond; B₄ is -O-; C₄ and D₄ are CH; R⁶⁷ is selected from the group consisting of hydrogen, a saccharide and a hydrophilic peptide; R⁶⁸ to R⁷⁹ are hydrogens; W⁶ and X⁶ may be the same or different and are selected from the group consisting of -C((CH₂)_{zz}OH)₂, C((CH₂)_{zz}CO₂H)₂ and C((CH₂)_{zz}NR_{pp}R_{pz})₂; Y⁶ and Z⁶ are the same and are selected from the group consisting of -(CH₂)_c-CO₂H, -CH₂-(CH₂-O-CH₂)_d-CH₂-CO₂H, and -(CH₂)_g-N(R¹⁴)-(CH₂)_h-CO₂H.
7. The method of claim 4 wherein said procedure utilizes light of wavelength in the region of 350-1300 nm.
8. The method of claim 4 wherein said diagnostic procedure is optical tomography.
9. The method of claim 4 wherein said diagnostic procedure is fluorescence endoscopy.
10. The method of claim 4 further comprising monitoring a blood clearance profile of said bioconjugate by fluorescence, absorbance or light scattering wherein light of wavelength in the region of 350-1300 nm is utilized.

11. The method of claim 4 wherein said procedure further comprises a step of imaging and therapy wherein said imaging and therapy is selected from the group consisting of absorption, light scattering, photoacoustic, and sonofluorescence technique.
- 5 12. The method of claim 4 wherein said procedure is for diagnosing atherosclerotic plaques and blood clots.
13. The method of claim 4 wherein said procedure further comprises administering localized therapy.
14. The method of claim 4 wherein said therapeutic procedure comprises
10 photodynamic therapy.
15. The method of claim 4 wherein said therapeutic procedure comprises laser assisted guided surgery (LAGS) for the detection of micrometastases.
16. A method for making the composition of claim 1 comprising conjugating the dyes to peptides or biomolecules by solid phase synthesis.
- 15 17. A method for preventing *in vivo* or *in vitro* fluorescence quenching by adding one to fifty percent biocompatible organic solvents to the diagnostic or therapeutic compositions of the dye molecules.
18. The method of claim 17 wherein said dye molecule is dissolved in a medium comprising one to 50 percent dimethyl sulfoxide (DMSO).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/01407

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 403/08; A61K 49/00

US CL : 548/427; 514/411; 424/9.1, 9.6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/427; 514/411; 424/9.1, 9.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE STRUCTURE SEARCH**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,453,505 A (LEE ET AL) 26 September 1995 (26.06.1995).	1-3
A	JP 59192595 A1 (TDK CORP.) 31 October 1984 (31.10.1984).	1-3

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 March 2001 (19.03.2001)

Date of mailing of the international search report

16 MAY 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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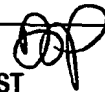
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PARALEGAL SPECIALIST



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/01407

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 1-16 (all in part), 17, and 18
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/01407

Continuation of Box I Reason 2: The variables A4, B4, C4, D4, X6, and W6 as defined in the claims yield a multitude of permutations of structures which cannot be readily classified. Consequently, claims 1-16 have been searched based on the classification of working examples that most closely resemble the claimed invention. Methods of claims 17 and 18 employ compositions of dye molecules of unspecified nature. The limitation that said dye molecules are contained in a diagnostic or therapeutic composition is of insufficient detail to commence a meaningful search of instant claims.

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